Environmental and Experimental Botany 155 (2018) 578–588

Contents lists available at ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Can elevated CO_2 buffer the effects of heat waves on wheat in a dryland cropping system?

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ARTICLE INFO

Keywords:
Climate change
Photosynthesis
Carbohydrate metabolism
Grain yield
Triticum aestivum L.
Grain quality

ABSTRACT

Increasing atmospheric CO_2 concentration [CO_2] drives the rise in global temperatures, with predictions of an increased frequency of heat waves (short periods of high temperatures). Both, CO_2 and high temperature, have profound effects on wheat growth and productivity. We tested whether elevated [CO_2] (eCO_2) has a potential to ameliorate the effects of simulated heat waves (HT) on wheat in a dryland cropping system. Wheat was field-grown at the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility under ambient [CO_2] (~390 ppm) or eCO_2 (~550 ppm) for two growing seasons, one with ample water supply and one of severe drought. Using heated chambers, heat waves (3-day periods of high temperatures) were imposed at critical growth stages before anthesis (HT1) or post-anthesis (HT2, HT3). Gas exchange, chlorophyll content and concentration of nitrogen (N) in mainstem flag leaves, as well as concentrations of stem water-soluble carbohydrates (WSC) in mainstems were monitored throughout the season. Yield, biomass and thousand kernel weights (TKW) were measured at maturity. Elevated [CO_2] moderated the effect on net CO_2 assimilation rates of pre-anthesis (HT1), but not of post-anthesis heat waves (HT2, HT3). Growth under eCO_2 increased stem WSC both, with and without experimental heat waves, but remobilisation decreased significantly under heat indicating that a greater WSC pool does not necessarily translate into greater remobilisation into the grain. Grain yield (g m^{-2}) was greater under eCO_2 and especially pre-anthesis heat stress decreased grain yield in the wetter season, and this decrease was stronger under eCO_2 (up to 20%) than under aCO_2 (up to 10%). Grain N decreased under eCO_2, but less so under heat stress. We conclude that eCO_2 may moderate some effects of heat stress in wheat but such effects strongly depend on seasonal conditions and timing of heat stress.

1. Introduction

Atmospheric carbon dioxide concentration [CO_2] has rapidly increased from a steady ~280 μmol mol^{-1} prior to the Industrial Revolution to currently ~405 μmol mol^{-1}, and is predicted to reach ~550 μmol mol^{-1} by the middle of this century (IPCC, 2014; Pearson and Palmer, 2000). This rise in [CO_2] drives an increase in global mean temperatures by 1.0 to 3.7 °C by the end of the 21st century. In addition, heat wave events (short periods of high temperatures) are likely to become more frequent and more severe (IPCC, 2014). Changes in global climate are already adversely affecting yield and quality of important food crops, such as wheat (*Triticum aestivum* L.), and are predicted to have more severe impacts in future climate scenarios (IPCC, 2014).

Aside from driving climate change, the increase in [CO_2] alone affects all plant systems (Ziska, 2008). CO_2 enrichment studies, in particular Free Air CO_2 Enrichment (FACE) experiments, have shown the following effects of elevated [CO_2] (eCO_2) on C_3 crops such as wheat: Greater net CO_2 assimilation rate but lowered stomatal conductivity and photorespiration (Ainsworth and Long, 2005; Kimball et al., 2001; Long et al., 2004); increased leaf and canopy water use efficiency and decreased transpiration (Drake et al., 1997; Leakey et al., 2009; Tausz-Posch et al., 2013a) as well as increases in dry matter accumulation and grain yield, mostly derived through increased fertile tiller numbers and sometimes also through increased single kernel weight (Ainsworth and Long, 2005; Dubey et al., 2015; Tausz-Posch et al., 2015). Elevated CO_2 can also lead to an increased accumulation of water-soluble

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https://doi.org/10.1016/j.envexpbot.2018.07.029
Received 17 January 2018; Received in revised form 22 June 2018; Accepted 29 July 2018
Available online 31 July 2018
0098-8472/

carbohydrates (WSC), such as starch and total non-structural carbohydrates in leaves and, in particular, fructans in the stems (Nie et al., 1995; Sild et al., 1999; Smart et al., 1994). In contrast, the concentration of mineral nutrients, particularly of nitrogen (N), is universally reduced under eCO_2 in vegetative and reproductive plant parts, translating directly to lower protein concentrations in vegetative tissues and grains, adversely affecting the nutritional and economic value of crops (Cotrufo et al., 1998; Taub and Wang, 2008).

With optimum growing temperatures between 17 and 23 °C (Shanmugam et al., 2013), wheat is very sensitive to heat (Farooq et al., 2011; Slafer and Rawson, 1995). Heat stress occurs when a plant is exposed to temperatures above an upper threshold for long enough to cause irreversible damage (Wahid et al., 2007). For wheat, threshold temperatures impacting growth and yield, are most commonly given between 31–35 °C (Barnabas et al., 2008; Ferris et al., 1998; Fischer, 2011), although some studies have reported high temperature impacts already above a threshold as low as 26 °C (Stone and Nicolas, 1994).

Heat stress decreases net CO_2 assimilation rates resulting in decreased photo-assimilate production as well as reduced dry-matter accumulation and yield (Bergkamp et al., 2018; Narayanan et al., 2015; Tahir and Nakata, 2005). When photosynthesis is constrained by heat stress, carbon (C) reserves, such as stem WSC (fructans) in wheat, can be used to fill the grains (Dreccer et al., 2013; Fokar et al., 1998). For example, the contribution of remobilised stem WSC to grain weight can increase from 10 to 20% under non-stress conditions to 30–50% under stress conditions (van Herwaarden et al., 2003; Wang et al., 2012). Similar trends were shown by Zamani et al. (2014) and Tahir and Nakata (2005) who reported that high temperature stress was correlated with increased WSC remobilisation from the mainstem into grains. Nevertheless, grain size and quality are affected by heat stress, with starch deposition into the grain being generally reduced under heat stress resulting in smaller kernels and more N per unit of starch (Stone and Nicolas, 1994; Farooq et al., 2011). Heat stress also induces phenological responses such as accelerated development, thus shortening the duration for the critical grain-filling period (Bergkamp et al., 2018; Stone and Nicolas, 1996).

The timing of heat stress during plant development is important because certain damage mechanisms only apply during particularly sensitive growth stages (Wollenweber et al., 2003). For example, in wheat heat stress shortly before or at anthesis will decrease grain numbers through floret abortion (Fischer, 1985), while stress episodes during the grain-filling stage will decrease grain weight by interfering with carbohydrate supply and translocation into the grains (Telfer et al., 2013).

Most previous research has focused on eCO_2 or heat stress separately, and those studies that investigated eCO_2 in combination with higher temperature commonly used moderate, continuous warming, not exceeding heat stress thresholds (e.g. de Oliveira et al., 2015). Little is known about the effects of heat waves, short periods of high temperatures, during critical developmental stages, in combination with eCO_2, despite the potential of eCO_2 to mitigate or interact with heat stress. For example, Shanmugam et al. (2013) reported that heat stress decreased net CO_2 assimilation rates in wheat, but when heat-stressed crops were grown under eCO_2, assimilation rates remained higher. Also, greater WSC pools under eCO_2 (Sild et al., 1999; Smart et al., 1994; Winzeler et al., 1990) may provide greater reserves for C remobilisation into grains, thereby ameliorating the negative effects of heat stress on grain weights.

The present study investigated whether growth under eCO_2 protects wheat from effects of heat waves in a dryland cropping system. Immediate and long-term responses to heat waves were recorded from pre-anthesis to maturity, and the experiment was replicated over two seasons. To account for different sensitive growth stages (Wollenweber et al., 2003), separate heat wave treatments were applied pre-anthesis (HT1) and during the grain-filling period (post-anthesis, HT2 and HT3). The experiment was conducted under ambient $[CO_2]$ (~390 ppm, aCO_2) and eCO_2 (~550 ppm) at the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility in Horsham, Victoria. This location is part of the semi-arid cropping region of the south-eastern Australian wheat belt, representative of globally important water-limited dryland wheat cropping areas, which are at particular risk from increased incidence and severity of climate change driven heat stress events (Sadras and Dreccer, 2015). Heat waves were simulated with mobile heat chambers (Nuttall et al., 2012), and treatment effects on grain yield assessed. Physiological (gas exchange and leaf chlorophyll) measurements were conducted in conjunction with assessments of stem WSC and leaf N concentrations from pre-anthesis through the grain-filling period and related to grain yield and grain N. These investigations were done on mainstems, because of their stable and relatively high contribution to grain yield as opposed to late-forming tillers which are affected by depleting photoassimilate supply (Darwinkel, 1978; Gan and Stobbe, 1995), even without environmental stresses.

In this study, we critically assess whether eCO_2 has the potential to ameliorate heat stress effects in wheat when grown in water-limited dryland wheat cropping systems. We determine if eCO_2-induced increases in net CO_2 assimilation will lead to a greater WSC pool in stems and, if yes, this helps to ameliorate the negative effects of heat stress on grain weights. Add-on effects for grain N are also investigated. In detail, we tested the following hypotheses:

1) Crops grown under eCO_2 have greater net assimilation rates than crops grown under aCO_2, both pre- and post-anthesis. Heat waves decrease net assimilation rates in crops grown under aCO_2, but eCO_2 buffers heat wave effects by maintaining net assimilation rates.
2) Increased net assimilation rates in crops grown under eCO_2 result in greater stem WSC concentrations. A greater pool of C reserves (WSC concentration) allows greater remobilisation to grains and this results in the maintenance of greater grain yield of eCO_2 grown crops subjected to heat waves.
3) Any reduction in grain N under eCO_2 is less in crops subjected to heat waves, because grains of heat stressed crops commonly have less carbohydrates relative to nitrogen.

2. Materials and methods

2.1. Site description

This experiment was conducted within the AGFACE facility during the 2013 and 2014 growing seasons. The research site of 7.5 ha is located 7 km west of Horsham, Victoria, Australia (36°45′07″S latitude, 142°06′52″E longitude, 128 m elevation); a semi-arid region of the Australian wheat belt. The average annual rainfall for the site is 435 mm with growing season (June-November) rainfall averaging 274 mm. The long term mean temperature is 16.5 °C. The soil type is classified as a Vertosol under the Australian Soil Classification (Isbell, 2002). This type of soil is characteristically pedal and non-dispersive on the surface. A detailed specification of the experimental facility can be found in Mollah et al. (2009). Local practices were the basis for agronomic management, including spraying of fungicides and herbicides, as needed.

2.2. Experimental setup

Eight rings (=experimental plots), arranged in four complete blocks, four under ambient CO_2 (aCO_2, ~390 μmol mol^{-1}) and four under elevated CO_2 (eCO_2, targeted concentration of ~550 μmol mol^{-1}), were used in both seasons. Ring diameters were 16 m in 2013 and 12 m in 2014. For the eCO_2 treatment, rings were surrounded with octagons of horizontal stainless steel tubes adjusted to about 0.15 m above the plant canopy at any given developmental stage. Pure CO_2 gas was injected in upwind direction through 0.3 mm laser-drilled holes facing outward of the ring. Using automated control

Table 1
Summary of experimental heatwave treatments (of 3 days duration) in this experiment: Control (no chamber, no heat), HT1 (pre-anthesis heatwave, starting 5 days before anthesis), HT2 (grain-filling heatwave, starting 15 days after anthesis), HT3 (late grain-filling heatwave, starting 30 days after anthesis). Letters indicate the parameters taken during each measurement or sampling day: "a" SPAD and gas exchange, "b" leaf nitrogen (N) and stem water-soluble carbohydrates (WSC), and "c" grain, straw and chaff dry weights and corresponding N measurements. Measurements and samplings during the heatwave treatments were taken on the third (last) day of each heatwave.

Year	Heat treatment	Days after anthesis (DAA)					
		−5	5	15	25	30	47
2013	Control	a, b	a	a, b	a	a, b	b, c
	HT1	a, b	a	a, b	a	a, b	b, c
	HT2			a, b	a	a, b	b, c
	HT3					a, b	b, c
2014	Control	a, b	a	a, b	a	a, b, c	
	HT1	a, b	a	a, b	a	a, b, c	
	HT2			a, b	a	a, b, c	

systems, enrichment of CO_2 to a central ring target concentration of 550 μmol mol^{-1} was maintained from sunrise to sunset, starting from 50% emergence. Detailed information about design and performance against targets of the injection system is given in (Mollah et al., 2009).

Within each ring, 4 m long × 1.44 m wide plots were randomly allocated. Within each plot, wheat (*Triticum aestivum* L. cultivar 'Yitpi') was sown into flatbeds at 0.27 m row spacing in both growing seasons. In each ring, four of these plots were randomly assigned to one of three heat wave treatments and one non-heated control in 2013, and in 2014, three plots were randomly assigned to one of two heat wave treatments and one control (Table 1). Cultivar Yitpi was chosen for this experiment because it is widely grown by farmers in the region. It is a semi-dwarf spring type wheat without significant vernalisation requirements. It has good early vigour and is best adapted to low to medium rainfall areas (Fitzgerald et al., 2016).

Heat waves were simulated with static heat chambers equipped with microclimate instruments monitoring internal air temperature and relative humidity. Heat chambers consisted of right-angle hollow section frame boxes (1200 mm width × 800 mm depth × 500 mm height) clad with Sun Tuff Greca Laserlite®, and were placed over a representative part of the plot for the duration of the heat wave treatment (3 days). The open base of the chambers allowed for mixing of outside air so that the chambers did not confound CO_2 concentration (Nuttall et al., 2012). The target temperature of 38 °C in the crop canopy was controlled by thermocouples. This target temperature was maintained during daylight hours and reduced to ambient temperatures during night time over the course of three days. This regime simulated the temperature conditions of the region under natural heat events (Talukder et al., 2013). A detailed description of the chamber construction and performance as well as pictures of the field set-up are given in Nuttall et al. (2012). By the end of the third day, heat chambers were removed from the experimental plot and crops resumed growth under normal field conditions.

Independent heat wave treatments applied were: pre-anthesis heat stress or HT1 (=heatwave 5 days before anthesis, with anthesis being defined as half of the spikes with anthers (DC 65), Zadoks et al., 1974), grain-filling heat stress or HT2 (=heatwave 15 days after anthesis), and late heat stress or HT3 (=heatwave 30 days after anthesis; not included in 2014 due to the very short growing season in that year), and there was also a non-heated control.

2.3. Data collection

In both years, grain yield, biomass and thousand kernel weights (TKW) were determined at maturity by harvesting all plants from representative lengths of the central four rows of the treated plot areas; with grain yield and biomass being expressed per m^2 ground area. In addition, grain weight per mainstem was determined on two randomly sampled mainstems per plot.

From pre-anthesis to maturity data were also collected by non-destructive field measurements (flag leaf gas exchange and chlorophyll, Table 1, letter a) and destructive sampling (biomass, flag leaf N, stem WSC and grain weight, Table 1, letters b and c) of mainstems. In 2013, for non-destructive field measurements, one random sample plant in each plot was tagged and the flag leaf measured repeatedly from the third (last) day of every heat treatment throughout the grain-filling period. In detail, chlorophyll and gas exchange for control and HT1 were measured 5 days before anthesis (-5) and 5, 15, 25, and 30 days after anthesis (DAA); for HT2, the measurement days were 15, 25, and 30 DAA; and for HT3, remaining measurement was at 30 DAA. At 30 DAA, some plants had senesced leaves which were excluded from measurements. Destructive samplings of two randomly chosen mainstems per plot per sampling date for biomass, N and WSC were undertaken as follows: For control and HT1, -5, 15, 30 and 47 DAA; for HT2, 15, 30 and 47 DAA; and for HT3, 30 and 47 DAA (Table 1). In 2014, the same protocol was followed, except that HT3 was omitted.

2.4. Gas exchange and chlorophyll

Gas exchange measurements were collected with an open path infrared gas analyser (LICOR-6400, LI−COR, Lincoln, NE, USA) with clear-top chamber ("sun + sky" cuvette, surface area dimension of 2 × 3 cm) or with integrated light source (Red/blue LED light source, 6400-02B). Measurements were taken during midday between 11:00am and 2:00pm (Houshmandfar et al., 2015) local time under light saturated conditions (at least 1200 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR)). The LICOR-6400 was set for an air flow rate of 500 μmol s^{-1}; and CO_2 was maintained at either 390 μmol mol^{-1} for aCO_2 plots or 550 μmol mol^{-1} for eCO_2 plots. Chamber and outside temperature were matched continuously and water vapour was scrubbed from the incoming air stream to ensure chamber relative humidity was close to ambient. In case a leaf did not cover the whole chamber area of 6 cm^2, the surface area was calculated as enclosed leaf length × leaf width in the chamber. Observations were logged automatically every 30 s using the instrument's "autolog" option for 2 min after rates stabilised (usually after 1 min). This protocol captures instantaneous photosynthesis and stomatal conductance (g_s) as close as possible to field conditions (Parsons et al., 1997; Tausz-Posch et al., 2013a).

Flag leaf chlorophyll content was monitored with a chlorophyll meter (SPAD 502, Konica Minolta, Macquarie Park, NSW, Australia). Five readings per leaf blade were averaged for each replicate.

2.5. Biomass

Two dominant mainstems were randomly sampled from each plot on each sampling day. Flag leaves (blades cut at the ligule) and stems (including leaf sheaths) were then removed, processed separately, and oven-dried at 70 °C for 72 h. Subsequently, samples were weighed for dry weight (DW) and ground using a plant grinding mill (FOSS TECATOR Cyclotec 1093, Thermo Fisher Scientific Inc., Waltham, MA, USA) in preparation for chemical analyses. For small samples, a mixer ball mill (Retsch Mixer Mill MM 400, Verder Scientific, Haan, Germany) was used. At maturity, mainstems were separated into straw (leaves and stems), grains, and chaff.

2.6. Nitrogen and WSC analysis

N concentration of wheat flag leaves was determined after combustion in an elemental analyser (LECO, TruMac, MI, USA). N remobilisation from flag leaves was calculated as the difference between the maximum N concentration and N concentration at maturity and

expressed as percentage.

WSC concentrations in stems were measured with an adapted anthrone method: 15 ± 0.5 mg of dried and ground stem material was extracted twice in 80% ethanol and twice in water (van Herwaarden et al., 2003; Talukder et al., 2013). For each extraction, sample material was thoroughly mixed with ethanol or water (VELP Scientifica Vortex Mixer, Usmate, Italy) and heated in a water bath (Thermoline Scientifica, Wetherill Park, NSW, Australia) at 84 °C for 60 min (ethanol) and 60 °C for 20 min (water). The extracts were then centrifuged (Eppendorf Centrifuge 5415D, Eppendorf AG, Hamburg, Germany) at 13,362 x g (RCF) for five min. 50 µL of the combined supernatants were mixed with 1 ml of anthrone reagent (0.2 g anthrone dissolved in 100 ml of 70% sulfuric acid) according to Trevelyan and Harrison (1952). As fructan is the most abundant WSC in wheat (Kaur et al., 2012; Xue et al., 2008; Zhang et al., 2013), D-fructose was used as standard. Fructose standards were prepared in an eight-fold serial 1:2 dilution from a stock of 1 mg mL^{-1} D-fructose in deionised water. Absorbance was read at 600 nm on a Tecan Sunrise microplate absorbance reader (Tecan Trading AG, Austria). WSC concentration was expressed as mg fructose equivalents per g DW. WSC remobilisation from stems was calculated by subtracting WSC concentrations at maturity from the maximum WSC concentration (usually reached 2–4 weeks after anthesis (Kiniry, 1993; Schnyder, 1993)).

2.7. Data analysis

Effects of CO_2, HT and their interactions were evaluated using R software (version 3.4.0; R Core Team, 2017) with RStudio (Version 1.0.143; RStudio Inc.). For data collected at maturity the linear mixed models R package lme4 (Bates et al., 2015) was used with fixed effects CO_2, heat treatment and year, with blocks and rings as random effects. Year was included as fixed rather than random effect because environmental conditions in both years were so contrasting that interactions with experimental treatments were of potential interest. As such interactions were rare, reported effects of heat treatments and interactions with CO_2 are regarded robust across contrasting conditions. For gas exchange parameters, SPAD, flag leaf N concentration, and stem WSC accumulation 'days after anthesis (DAA)' was used as an additional within-plot factor. As in these models DAA and/or interactions with DAA had strong effects, each DAA was subsequently analysed separately. P-values were obtained with the R package lmerTest (Kuznetsova et al., 2017). Visual inspections of residual plots were done on all models and fits were considered adequate. Graphs were produced using "ggplot2″ package (Wickham, 2009) of R software (v 3.0.3) (R Core Team, 2017). Statistical effects were reported at $p < 0.100$, following the suggestions for FACE experiments by Filion et al. (2000).

3. Results

3.1. CO_2, weather and phenology

The average seasonal daytime CO_2 concentrations achieved in 2013 was 545 ± 42 ppm (mean ± SD) for eCO_2 and 393 ± 16 ppm for aCO_2 treatment. In 2014, seasonal daytime CO_2 concentrations averaged 551 ± 55 ppm for eCO_2 and 393 ± 26 ppm for aCO_2.

In 2013 and in 2014, the experimental field site received 324 and 115 mm of rainfall during the wheat growing season, respectively, compared to the long term in-growing season average of 274 mm. Rainfall was extremely low in 2014 to the extent that many local farmers had to abandon their crops mid-season. Therefore, the site was supplementally irrigated with an additional 34 mm of water pre-season and 94 mm during the growing season to keep crops alive. In 2013, an above-average rainfall year, no supplemental irrigation was applied. Average maximum temperature was 21.6 °C in 2013 and 22.5 °C in 2014. Anthesis occurred 143 days after sowing in 2013 and 130 days after sowing in 2014. The grain-filling period was 47 days in 2013 and 30 days in 2014 (Fig. 1).

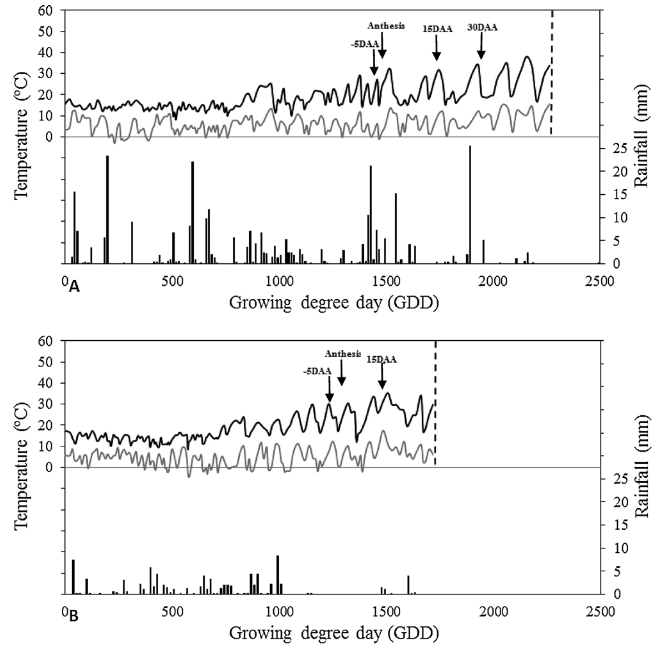

Fig. 1. Daily minimum ambient temperature (°C, grey line), daily maximum ambient temperature (°C, black line) and rainfall (mm, black columns) at the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility from sowing of wheat until maturity in 2013 (A) and 2014 (B), as a function of growing degree days (GDD). Downward arrows indicate heatwave treatments. HT1 was applied 5 days before anthesis (-5DAA), HT2 15 days after anthesis (15DAA), and HT3 30 days after anthesis (30DAA). Anthesis in 2013 was at 1478 GDD, while in 2014 anthesis was earlier at 1264 GDD.

In 2013, average temperatures inside the heat chambers were 34 °C during HT1, 37 °C during HT2 and 37 °C during HT3, compared to ambient air temperatures from control plots of 22 °C, 28 °C and 25 °C, respectively. In 2014, heat wave treatments saw 37 °C during HT1 and 38 °C during HT2, compared to 22 °C and 31 °C, respectively, for the controls (Fig. 2). Correspondingly, relative humidity was lower and vapour pressure deficit was greater in heat chambers (Fig. 2). There were also naturally occurring heat stress days in both years. In 2013, days above 30 °C occurred on two days during early grain-filling (2 and 18 days after anthesis (DAA)) as well as on seven days during the final phase of grain-filling (between 30 and 45 DAA). In 2014, naturally occurring heat stress events occurred before anthesis (one day), increasing in frequency shortly after (8 days throughout the grain-filling period; between 4 and 30 DAA, Fig. 1).

3.2. Biomass, grain yield and corresponding N concentrations

Grain yield was stimulated by eCO_2 and biomass closely followed this trend (Table 2). A significant CO_2 x year x heat interaction was caused by a yield decrease in eCO_2-grown wheat after pre-anthesis heat stress (HT1) in the wetter year. There was no significant CO_2 effect ($p = 0.110$), but a significant heat stress effect on thousand kernel weight (TKW), whereby TKW were decreased especially in response to late heat stress; HT3 in 2013, but also HT2 in 2014. Grain yield per mainstem decreased especially in response to HT2, except for the dry year under eCO_2 (interaction CO_2 x year x heat). Straw and chaff [N] remained unaffected by heat or CO_2, but the eCO_2-related decrease in grain [N] was slightly less in heat treated plants than non-heated controls (interaction CO_2 x heat).

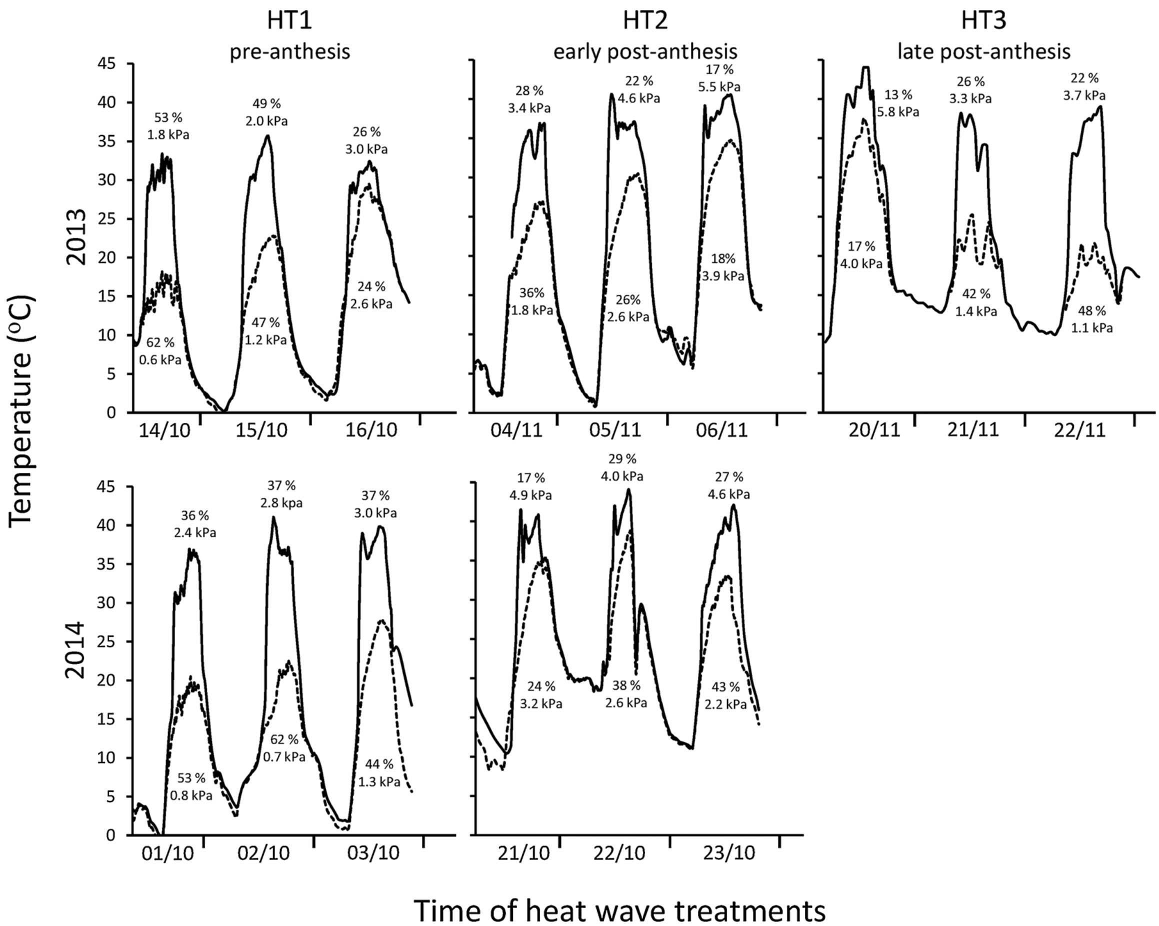

Fig. 2. Heat wave treatments applied to wheat (*cv.* Yitpi) within the AGFACE facility. High temperature treatments (solid line) were applied in 2013 and 2014, at 5 days pre-anthesis (HT1) and 15 days post-anthesis (HT2). For 2013, treatments were also applied at 30 days post-anthesis (HT3). Ambient air temperature (dashed line) is also presented. Annotated values are canopy relative humidity (%) and vapour pressure deficit (kPa) within the chamber and ambient air equivalent for the period the treatment was applied on a daily interval.

3.3. Flag leaf chlorophyll

Flag leaf chlorophyll (SPAD readings; Fig. 3) declined from two weeks past anthesis. Heat generally depressed SPAD values and upon HT1 more so under eCO_2.

3.4. Gas exchange

Over the post-anthesis decline of net assimilation rates, eCO_2 generally increased net assimilation rates, on average by 64% in 2013 (Fig. 4A and B) and 54% in 2014 (Fig. 4C and D). Heat wave treatments also affected net assimilation rates: Net assimilation rates were greatest in eCO_2 grown heat-stressed plants on the last day of HT1 heatwave treatment (significant interaction HT x CO_2, Fig. 4A and C), but afterwards, heat-treated plants tended to have lower assimilation rates under eCO_2 (interaction HT x CO_2). In contrast, after heatwave treatment applied during grain-filling (HT2), plants had generally decreased net assimilation rates (Fig. 4B and D) especially under eCO_2 (CO_2 x heat interaction, Fig. 4B), indicating that eCO_2 grown control plants maintained the greatest net assimilation rates. The heatwave treatment applied at 30 DAA had no effect on net assimilation rates (data not shown).

There were few effects of heatwave treatments and eCO_2 on g_s; heatwave treatments appeared to suppress a late (25 DAA) increase in g_s under eCO_2 (Fig. 4E–H).

3.5. Flag leaf N

Flag leaf N concentrations declined from anthesis until maturity (Fig. 5A–C in 2013, Fig. 5D–E in 2014) and, where CO_2-effects were significant they showed lower N concentrations under eCO_2 (Fig. 5A, C and D) and after the pre-anthesis heatwaves (Fig. 5A and C). Remobilisation of flag leaf N (calculated on a flag leaf concentration basis; Table 3) was not affected by heatwave treatments or CO_2.

3.6. Stem WSC

Stem WSC concentrations generally declined from about two weeks after anthesis until maturity (Fig. 6) and eCO_2 increased stem WSC concentrations generally across both years (whereby effects were sometimes stronger in 2013, as shown by significant interactions with year). Where heatwave effects on WSC were significant, they decreased stem WSC concentrations, and in some cases more for eCO_2 grown plants, and more pronounced in 2013 (shown by interactions with CO_2 and year (Fig. 6). Elevated CO_2 grown control plants had generally the greatest WSC concentrations. The late heat wave treatment (HT3) had no significant effect on WSC. Remobilisation of stem WSC decreased significantly in plants subjected to the heatwave treatments, both for HT1 and HT2 (Table 3), and there was no interaction with year or CO_2.

4. Discussion

In this study, the use of the AGFACE facility and purpose-built static

Table 2
Harvest (maturity) grain yield, aboveground biomass, and thousand kernel weight (TKW) assessed on a whole plant basis, and grain yield per mainstem and N concentrations of grain, straw and chaff ([N], in % DW) assessed on mainstems of wheat (*Triticum aestivum* cv. 'Yitpi') grown in the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility during 2013 and 2014. Heatwave treatments: Control; naturally occurring temperatures. HT1 heat treatment with heat chambers for three days, starting five days before anthesis (DC65); HT2 three days heatwave starting 15 days post-anthesis; HT3 three days heatwave starting 30 days post-anthesis. CO_2 treatments: ambient [CO_2] (aCO_2, ~390 μmol mol^{-1}) and elevated [CO_2] (eCO_2, ~550 μmol mol^{-1}). Each value is the mean ± SE of n = 4. P-values for Heat, CO_2, and year. ns $P \geq 0.100$. DW dry weight.

Year	CO_2	Heat	Grain yield [g m^{-2}]	Biomass [kg m^{-2}]	TKW [g]	Grain yield [g mainstem^{-1}]	Straw [N] %	Chaff [N] %	Grain [N] %
2013	aCO_2	Control	674 ± 65	1.68 ± 0.14	37.2 ± 2.8	3.97 ± 0.29	0.40 ± 0.06	0.45 ± 0.07	1.86 ± 0.06
		HT1	670 ± 56	1.68 ± 0.12	37.9 ± 1.5	3.43 ± 0.21	0.28 ± 0.02	0.36 ± 0.02	1.70 ± 0.06
		HT2	619 ± 29	1.63 ± 0.07	35.6 ± 2.9	3.31 ± 0.18	0.29 ± 0.03	0.39 ± 0.03	1.76 ± 0.08
		HT3	649 ± 61	1.75 ± 0.17	34.1 ± 2.2	3.39 ± 0.14	0.36 ± 0.03	0.47 ± 0.04	1.87 ± 0.12
	eCO_2	Control	843 ± 93	2.07 ± 0.19	42.9 ± 1.6	3.91 ± 0.25	0.24 ± 0.04	0.34 ± 0.04	1.54 ± 0.09
		HT1	678 ± 48	1.74 ± 0.11	42.0 ± 3.1	3.91 ± 0.05	0.27 ± 0.03	0.32 ± 0.02	1.62 ± 0.03
		HT2	772 ± 43	1.90 ± 0.07	42.9 ± 1.3	3.09 ± 0.29	0.24 ± 0.02	0.40 ± 0.03	1.58 ± 0.04
		HT3	766 ± 40	2.01 ± 0.10	39.5 ± 2.6	3.53 ± 0.29	0.22 ± 0.03	0.33 ± 0.04	1.42 ± 0.10
2014	aCO_2	Control	126 ± 13	0.35 ± 0.04	34.3 ± 3.7	0.87 ± 0.05	0.35 ± 0.16	0.78 ± 0.13	2.43 ± 0.28
		HT1	130 ± 13	0.37 ± 0.04	36.5 ± 3.0	0.78 ± 0.07	0.40 ± 0.15	0.70 ± 0.15	2.38 ± 0.21
		HT2	127 ± 19	0.41 ± 0.06	31.9 ± 4.2	0.71 ± 0.08	0.41 ± 0.14	0.75 ± 0.13	2.50 ± 0.17
	eCO_2	Control	194 ± 35	0.60 ± 0.09	37.2 ± 1.9	0.81 ± 0.09	0.31 ± 0.07	0.60 ± 0.06	2.10 ± 0.21
		HT1	227 ± 55	0.71 ± 0.19	39.1 ± 2.4	0.91 ± 0.13	0.30 ± 0.03	0.60 ± 0.09	2.45 ± 0.30
		HT2	172 ± 30	0.60 ± 0.11	33.2 ± 1.8	1.00 ± 0.09	0.37 ± 0.10	0.73 ± 0.11	2.40 ± 0.23
Statistics		CO_2	P = 0.033	P = 0.022	ns	ns	ns	ns	ns
		Heat	ns	ns	P = 0.004	P = 0.007	ns	ns	ns
		Year	P < 0.001	P < 0.001	P = 0.085	P < 0.001	ns	P < 0.001	P < 0.001
		CO_2 x Heat	ns	ns	ns	ns	ns	ns	P = 0.074
		CO_2 x Year	ns	ns	ns	ns	ns	ns	ns
		Heat x Year	ns	ns	P = 0.088	P = 0.001	ns	ns	ns
		CO_2 x Year x Heat	P = 0.069	ns	ns	P < 0.069	ns	ns	ns

heat chambers simulated environments likely to be encountered by wheat crops in semi-arid cropping regions under projected future climate. The experiment was conducted over two contrasting seasons. In 2013, an above-average rainfall year, a longer grain-filling duration provided sufficient time for greater grain yield combined with lower grain N concentration. In contrast, in 2014, a low-yielding season with generally higher temperatures and low water availability, the crop matured early resulting in low yields but greater grain N concentration, typical for between-season variations at this site (Houshmandfar et al., 2016).

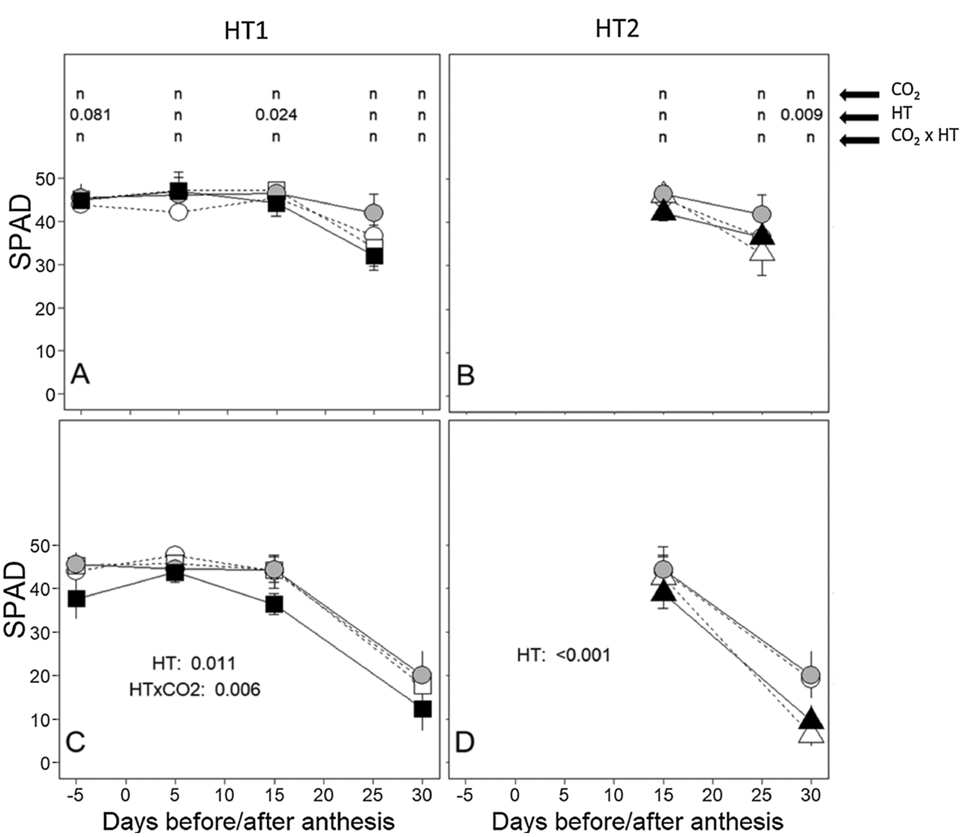

Fig. 3. Chlorophyll (SPAD reading; arbitrary unit) of mainstem flag leaves of *Triticum aestivum* L. cv. Yitpi grown under two CO_2 concentrations: elevated (~550 μmol mol^{-1}, solid black and grey symbols, continuous line) and ambient (~390 μmol mol^{-1}, open symbol, dashed line); and subjected to short-term (3 days) heatwave treatments: control (no heat treatment; circle symbol), pre-anthesis heatwave or HT1 (applied 5 days before anthesis, square symbol), grain-filling heat stress or HT2 (applied 15 days after anthesis (DAA), triangle symbol) during 2013 (A, B) and 2014 (C, D) field experiments. Fig. 2A and C show comparison of HT1 against control while Fig. 2B and D show HT2 against control comparison. Each symbol represents the mean ± SE of four replicate plots. Location of *P*-values in each graph: above each DAA indicates significance of CO_2 treatment (CO_2), heat treatment (HT) and their interaction (CO_2 x HT) at each sampling date across both years; effects of year were significant in most cases and are not reported, but any significant (P < 0.100) interactions with year are reported. Overall effects of an analysis using DAA as repeated measurement factor are reported in the middle of the graph; *P*-values for effects of heat treatment, CO_2 and their interactions are shown where P < 0.100. DAA and interactions with DAA were mostly significant but are not listed.

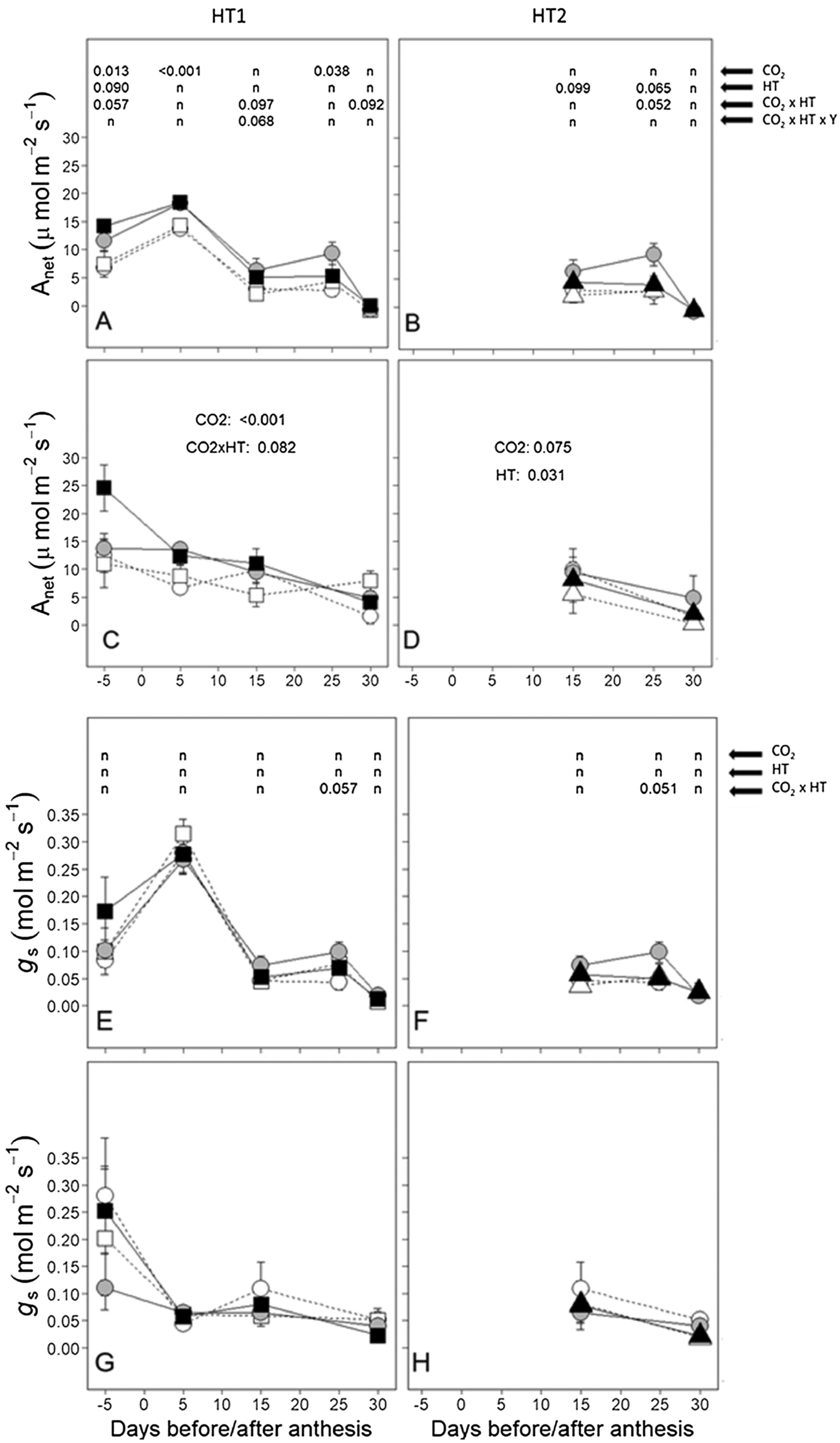

Fig. 4. Net assimilation rates (A_{net}, 3A- 3D) and stomatal conductance (g_s, 3E- 3 H) of mainstem flag leaves of *Triticum aestivum* L. cv. Yitpi grown under two CO_2 concentrations: elevated (~550 μmol mol^{-1}, solid black and grey symbols, continuous line) and ambient (~390 μmol mol^{-1}, open symbol, dashed line); and subjected to short-term (3 days) heatwave treatments: control (no heat; circle symbol), pre-anthesis heat stress or HT1 (applied 5 days before anthesis, square symbol), grain-filling heat stress or HT2 (applied 15 days after anthesis (DAA), triangle symbol) during 2013 (A, B, E, F) and 2014 (C, D, G, H) field experiments. Fig. 3A, C, E, and F show comparison of HT1 against control while Fig. 3B, D, F, and H show HT2 against control comparison. Each symbol represents the mean ± SE of four replicate plots. Location of *P*-values in each graph: above each DAA indicates significance of CO_2 treatment (CO_2), heat treatment (HT) and their interaction (CO_2 x HT) at each sampling date across both years; effects of year were significant in most cases and are not reported, but any significant ($P < 0.100$) interactions with year are reported. Overall effects of an analysis using DAA as repeated measurement factor are reported in the middle of the graph; *P*-values are shown for effects of heat treatment, CO_2 and their interactions where $P < 0.100$. DAA and interactions with DAA were mostly significant but are not listed.

4.1. Effects of heat and eCO_2 on gas exchange rates, WSC and grain weight

In accordance with hypothesis one, and previous reports from the same site (Tausz-Posch et al., 2013a, 2013b; Thilakarathne et al., 2015) as well as global meta-analyses of FACE experiments (Ainsworth and Long, 2005; Leakey et al., 2009), crops grown under eCO_2 had generally greater net CO_2 assimilation rates throughout the growing season, particularly under control (not heat-stress) conditions.

Heatwave treatments generally decreased net CO_2 assimilation rates. There are direct and indirect effects of high temperature that can

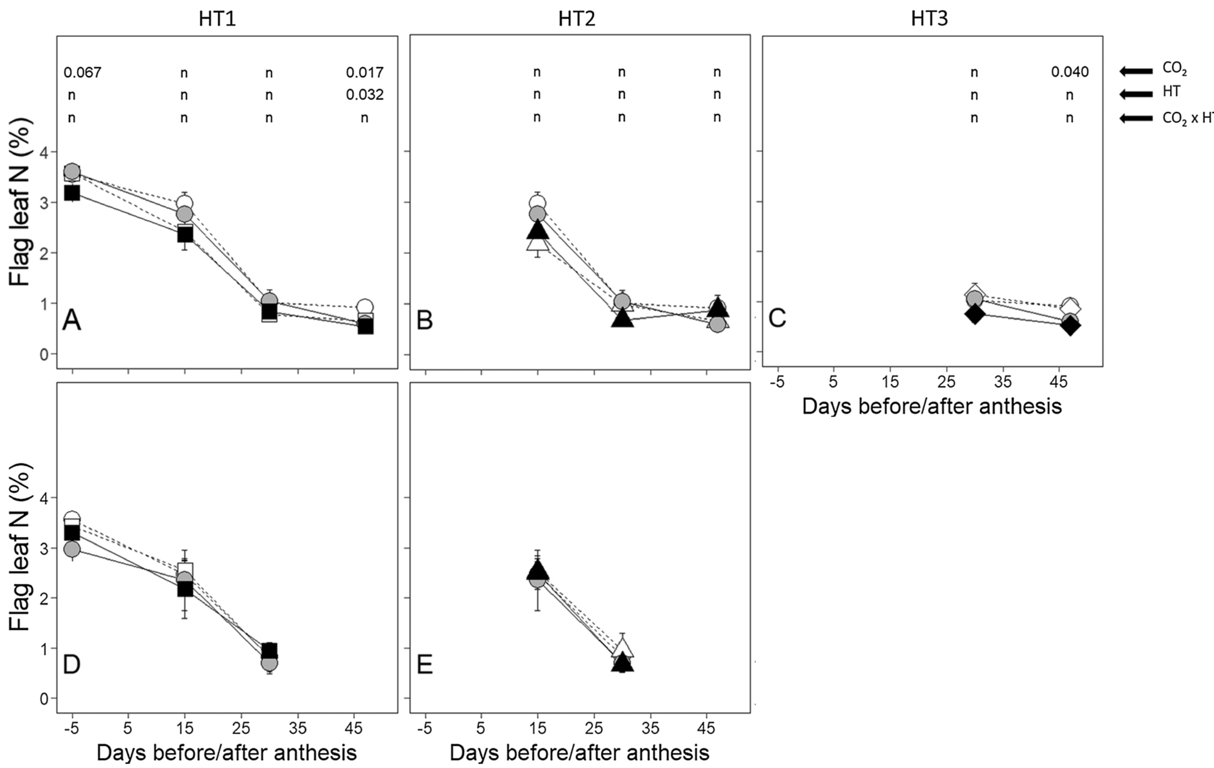

Fig. 5. Flag leaf nitrogen (N) concentration (% DW) of mainstem flag leaves of *Triticum aestivum* L. cv. 'Yitpi' grown under two CO_2 concentrations: elevated (~550 μmol mol^{-1}, solid black and grey symbols, continuous line) and ambient (~390 μmol mol^{-1}, open symbol, dashed line); and subjected to short-term (3 days) heatwave treatments: control (no heat; circle symbol), pre-anthesis heatwave or HT1 (applied 5 days before anthesis, square symbol), grain-filling heatwave or HT2 (applied 15 days after anthesis (DAA), triangle symbol), and late heatwave or HT3 (applied 30 DAA, diamond symbol) during 2013 (A, B, C) and 2014 (C, D) field experiments. Fig. 4A and D show comparison of HT1 against control, Fig. 4B and E show HT2 against control comparison, and Fig. 4C show HT3 against control. Each symbol represents the mean ± SE of four replicate plots. Location of *P*-values in each graph: above each DAA indicates significance of CO_2 treatment (CO_2), heat treatment (HT) and their interaction (CO_2 x HT) at each sampling date across both years; effects of year were significant in most cases and are not reported, but any significant ($P < 0.100$) interactions with year are reported. Overall effects of an analysis using DAA as repeated measurement factor are reported in the middle of the graph; *P*-values are shown for effects of heat treatment, CO_2 and their interactions where $P < 0.100$. DAA and interactions with DAA were mostly significant but are not listed.

limit the CO_2 assimilation in C3 plants. Direct effects include, for example, the stimulation of photorespiration over photosynthesis or inhibition of reactions downstream of PSII (Sharkey, 2005). Indirect effects can result from decreased stomatal conductance as a response to increased VPD. The consequence of closing stomata is a lower internal CO_2 concentration (Ci) through diffusional limitations leading to decreases in photosynthesis in turn (Perez-Martin et al., 2009). In the current study stomatal conductance was hardly affected by heat or CO_2 treatments and as such it is unlikely that indirect effects of high temperature via VPD negatively affected photosynthetic rates. A similar result was found by Rashid et al. (2018) who reported that, regardless of VPD, heat impaired the photosynthetic capacity in wheat by biochemical limitations while diffusional limitations were insignificant.

A buffer effect of eCO_2 against heatwave impacts was only found in direct response to a pre-anthesis heatwave (HT1) when eCO_2 grown crops had increased net CO_2 assimilation rates on the third day of heatwave exposure. Similarly, Shanmugam et al. (2013) reported greater net assimilation rates in eCO_2 grown wheat during a three-day heat stress event prior to anthesis. In contrast, there was no such ameliorating effect of eCO_2 when the heatwave treatment was applied during grain-filling (HT2), with net CO_2 assimilation rates generally decreasing under heat. This indicates that any amelioration of

Table 3
Remobilisation of nitrogen (N) from flag leaves (% of flag leaf DW) and water-soluble carbohydrates (WSC) from stems (mg g^{-1} stem DW) during grain-filling of wheat (*Triticum aestivum* cv. 'Yitpi') grown in the Australian Grains Free Air CO_2 Enrichment (AGFACE) facility in Horsham, Victoria, Australia. Wheat was grown either under aCO_2 (~390 ppm) or eCO_2 (~550 ppm) and subjected to only ambient weather conditions (Control), pre-anthesis heatwave (HT1, starting 5 days before anthesis) or grain-filling heatwave (HT2, starting 15 days after anthesis). Each heatwave treatment lasted 3 days. Means ± SE of n = 4 replicate plots.

	Control		HT1		HT2	
	aCO_2	eCO_2	aCO_2	eCO_2	aCO_2	eCO_2
Remobilised N (% flag leaf DW)[a]						
2013	2.6 ± 0.1	3.0 ± 0.1	2.9 ± 0.2	2.6 ± 0.2	2.9 ± 0.1	2.7 ± 0.4
2014	2.7 ± 0.2	2.3 ± 0.3	2.6 ± 0.2	2.4 ± 0.2	2.3 ± 0.1	2.3 ± 0.2
Remobilised WSC (mg g^{-1} stem DW)[b]						
2013	220 ± 10	214 ± 25	204 ± 8	192 ± 13	210 ± 15	166 ± 16
2014	195 ± 23	244 ± 41	174 ± 33	170 ± 42[b]	195 ± 37	162 ± 40

[a] The effects of year on remobilised N was significant at $P = 0.013$, all other effects on N remobilisation were not significant ($P > 0.100$).
[b] The effect of heat (HT1 and HT2) on remobilised WSC was significant at $P = 0.011$, all other effects on WSC remobilisation were not significant ($P > 0.100$).

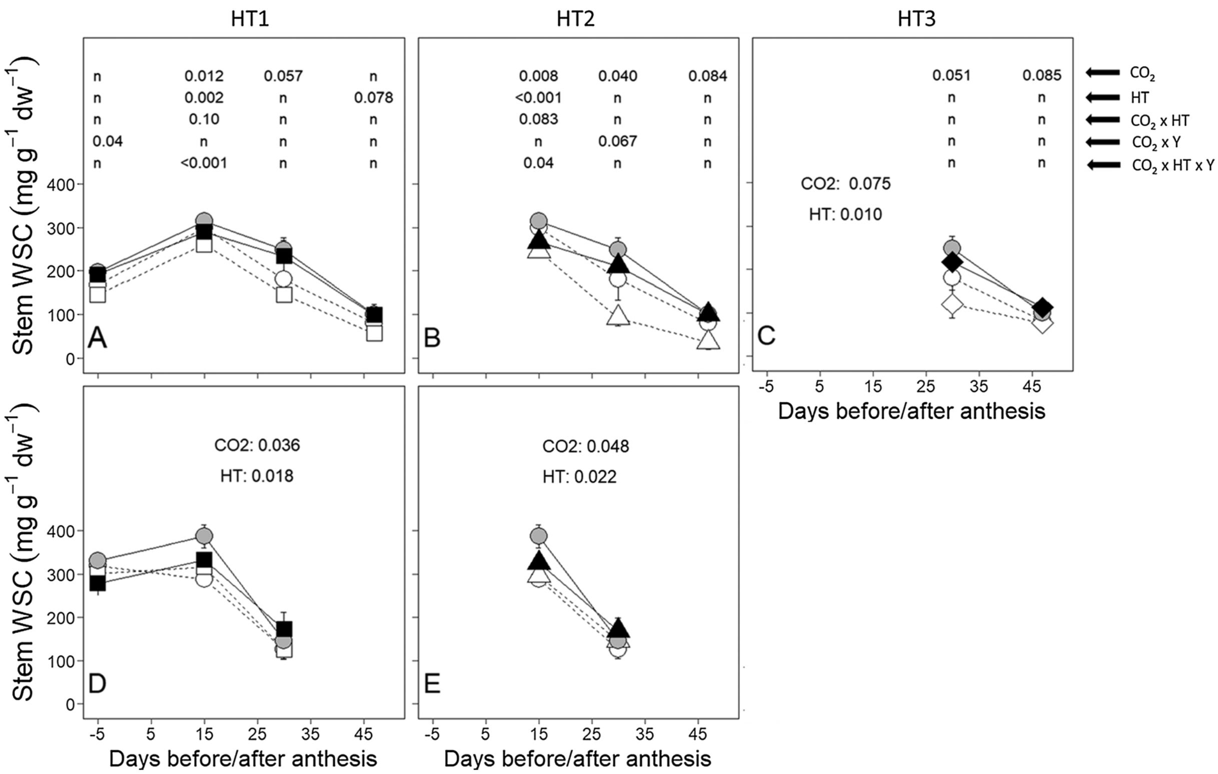

Fig. 6. Stem water-soluble carbohydrate (WSC) concentration (mg g^{-1} DW) of mainstem flag leaves of *Triticum aestivum* L. cv. Yitpi grown under two CO_2 concentrations: elevated (~550 μmol mol^{-1}, solid black and grey symbols, continuous line) and ambient (~390 μmol mol^{-1}, open symbol, dashed line); and subjected to short-term (3 days) heatwave treatments: control (no heat; circle symbol), pre-anthesis heatwave or HT1 (applied 5 days before anthesis, square symbol), grain-filling heatwave or HT2 (applied 15 days after anthesis (DAA), triangle symbol), and late heatwave or HT3 (applied 30 DAA, diamond symbol) during 2013 (A, B, C) and 2014 (C, D) field experiments. Fig. 6A and D show comparison of HT1 against control, Fig. 6B and E show HT2 against control comparison, and Fig. 6C show HT3 against control. Each symbol represents the mean ± SE of four replicate plots. Location of P-values in each graph: above each DAA indicates significance of CO_2 treatment (CO_2), heat treatment (HT) and their interaction (CO_2 x HT) at each sampling date across both years; effects of year were significant in most cases and are not reported, but any significant ($P < 0.100$) interactions with year are reported. Overall effects of an analysis using DAA as repeated measurement factor are reported in the middle of the graph; P-values are shown for effects of heat treatment, CO_2 and their interactions where $P < 0.100$. DAA and interactions with DAA were mostly significant but are not listed.

heatwave effects by eCO_2 is strongly dependent on developmental and/or environmental conditions.

In agreement with the literature on the effect of eCO_2 on stem carbohydrate accumulation in wheat (Sild et al., 1999; Smart et al., 1994; Winzeler et al., 1990), and in partial agreement with hypothesis 2, our study showed a consistent increase in stem WSC concentration under eCO_2 in both, control plants and plants subjected to experimental heatwaves. However, this greater pool of stem carbohydrate reserves under eCO_2 did not result in a buffer effect against heatwaves through greater remobilisation. In contrast, heatwave treatment even decreased WSC remobilisation. This is in apparent contrast to Tahir and Nakata (2005) who found that wheat crops remobilised relatively more WSC (240 mg g^{-1} DW) than control plants (170 mg g^{-1} DW) in response to heat stress. It is noteworthy that the heat stress study by Tahir and Nakata (2005) was conducted in a controlled environment where plants were watered daily. This is in stark contrast to our study where crops grew under terminal drought conditions in the field. Davidson and Chevalier (1992) demonstrated a strong dependence of WSC remobilisation on water availability and reported that irrigated wheat crops remobilised approximately three times more WSC than non-irrigated plants. This might indicate that whilst stems are important storage sites for reserve carbohydrates, the remobilisation of these reserves is strongly affected by environmental factors. Our results show that a mitigation of heat stress effects through increased carbohydrate reserves under eCO_2 is not necessarily granted.

Grain yield (g m^{-2}) was stimulated by eCO_2 in both, control and heat stressed plots whilst there was no consistent CO_2 effect on yield per mainstem. Elevated [CO_2] generally stimulates grain yield in wheat (Ainsworth and Long, 2005; Fitzgerald et al., 2016; Tausz-Posch et al., 2015), but it is known that this is mostly through increased stem and spike numbers rather than increased grain weights per stem (McMaster et al., 1999; de Oliveira et al., 2015; Tausz-Posch et al., 2015).

Area-based grain yield was mainly affected by pre-anthesis heat stress, whereas TKW was mainly affected by post-anthesis heat stress, consistent with effects on grain set by pre-anthesis heat stress (Fischer, 1985) and effects on grain weight by heat stress during grain filling, when it restricts carbohydrate supply and translocation into the grains (Telfer et al., 2013). Interactions between year, heat and CO_2 seemed to play out differently for area based yields and yields per mainstem: On an area basis, the effect of heat seemed stronger under eCO_2 (10–20%) than under aCO_2 (1–10%), yet on a mainstem basis, the effect of heat stress on yield was greater under aCO_2 (10–20%) than eCO_2 (up to 10%). This apparent discrepancy indicates not only differential responses of main and secondary tillers to heat stress (as also found in Sun et al., 2018), but also to potential interactions with CO_2.

4.2. Effects of heatwave and CO_2 treatment on biomass N and grain N

More than 80% of total aboveground N in wheat accumulates until anthesis, and this can account for up to 100% of the total N content in wheat grains (Tahir and Nakata, 2005). Grain N accumulation depends on the remobilisation of previously accumulated N in different plant organs (Palta and Fillery, 1995) with flag leaves playing a significant role in this process contributing at least 24% of all grain N (Simpson

et al., 1983). The current study followed flag leaf N concentrations and flag leaf N remobilisation from anthesis to maturity and also assessed straw, chaff and grain N concentrations in response to growth under eCO_2 and superimposed heatwaves. N concentrations in flag leaves and grains were negatively affected by eCO_2 treatment consistent with previous studies (e.g. Erbs et al., 2010; Buchner et al., 2015) but the eCO_2-induced decrease in grain N was slightly less in heat-treated crops than non-heated controls. Our finding is in agreement with Daniel and Triboi (2000) and Uhlen et al. (1998) who reported that grain N is less affected by heat than grain C. Starch deposition in grains is generally reduced under heat stress resulting in more N per unit of starch (Farooq et al., 2011; Stone and Nicolas, 1994). Grain N followed this trend under eCO_2 but an interaction with heat stress ameliorated this effect. Overall, these findings support our third hypothesis, where we suggested that the eCO_2-induced reduction in grain N would be less pronounced in heat-stressed crops.

5. Conclusion

From this study on wheat grown in a dryland cropping system there is some evidence for a general buffering effect of eCO_2 against (experimentally imposed) heatwaves. However, results strongly depended on timing of heatwaves as well as environmental conditions during the growing season. An apparent buffering effect of growth under eCO_2 against heatwave impacts was found for instantaneous net CO_2 assimilation rates in direct response to a pre-anthesis heatwave, when eCO_2 grown crops had high rates during the heatwave. In contrast, despite greater stem WSC reserves in eCO_2 grown plants, C remobilisation into the grain was not increased, resulting in decreased grain yield per mainstem, especially after heatwaves during the critical grain-filling period. This result is in contrast to reports of increased WSC remobilisation under heat stress observed in well-watered crops, and demonstrates the strong dependence of remobilisation processes on environmental growing conditions. Finally, reductions in grain N under eCO_2 were less pronounced under heat stress. We conclude that eCO_2 may moderate some effects of heat stress on grain yield and that eCO_2-induced reductions in grain N are less pronounced under heat stress, but this study also shows that such effects strongly depend on seasonal conditions and timing of heat stress.

Acknowledgements

The Australian Grains Free Air CO_2 Enrichment (AGFACE) program is jointly run by The University of Melbourne and Agriculture Victoria Research with funding from the Grains Research and Development Corporation and the Australian Commonwealth Department of Agriculture and Water Resources. The authors gratefully acknowledge Mahabubur Mollah (Agriculture Victoria Research) for running the CO_2 enrichment technology and Russel Argall (Agriculture Victoria Research) and the field team for agronomic trial management.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.envexpbot.2018.07.029.

References

Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO2 enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO2. New Phytol. 165 (2), 351–372.
Barnabas, B., Jager, K., Feher, A., 2008. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 31 (1), 11–38.
Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1–48.
Bergkamp, B., Impa, S.M., Asebodo, A.R., Fritz, A.K., Jagadish, S.V.K., 2018. Prominent winter wheat varieties response to post-flowering heat stress under controlled chambers and field based heat tents. Field Crops Res. 222, 143–152.
Buchner, P., Tausz, M., Ford, R., Leo, A., Fitzgerald, G.J., Hawkesford, M.J., Tausz-Posch, S., 2015. Expression patterns of C- and N-metabolism related genes in wheat are changed during senescence under elevated CO_2 in dry-land agriculture. Plant Sci. 236, 239–249.
Cotrufo, M.F., Ineson, P., Scott, A., 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. Glob. Change Biol. 4 (1), 43–54.
Daniel, C., Triboi, E., 2000. Effects of temperature and nitrogen nutrition on the grain composition of winter wheat: effects on gliadin content and composition. J. Cereal Sci. 32 (1), 45–56.
Darwinkel, A., 1978. Patterns of tillering and grain production of winter-wheat at a wide-range of plant densities. Netherlands J. Agric. Sci. 26 (4), 383–398.
Davidson, D.J., Chevalier, P.M., 1992. Storage and remobilization of water-soluble carbohydrates in stems of spring wheat. Crop Sci. 32 (1), 186–190.
de Oliveira, E.A.D., Siddique, K.H.M., Bramley, H., Stefanova, K., Palta, J.A., 2015. Response of wheat restricted-tillering and vigorous growth traits to variables of climate change. Glob. Change Biol. 21 (2), 857–873.
Drake, B.G., Gonzalez-Meler, M.A., Long, S.P., 1997. More efficient plants: A consequence of rising atmospheric CO_2? Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 609–639.
Dreccer, M.F., Chapman, S.C., Rattey, A.R., Neal, J., Song, Y.H., Christopher, J.T., Reynolds, M., 2013. Developmental and growth controls of tillering and water-soluble carbohydrate accumulation in contrasting wheat (*Triticum aestivum L.*) genotypes: can we dissect them? J. Exp. Bot. 64 (1), 143–160.
Dubey, S.K., Tripathi, S.K., Pranuthi, G., 2015. Effect of elevated CO_2 on wheat crop: mechanism and Impact. Crit. Rev. Environ. Sci. Technol. 45 (21), 2283–2304.
Erbs, M., Manderscheid, R., Jansen, G., Seddig, S., Pacholski, A., Weigel, H.J., 2010. Effects of free-air CO_2 enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley grown in a crop rotation. Agric. Ecosyst. Environ. 136, 59–68.
Farooq, M., Bramley, H., Palta, J.A., Siddique, K.H.M., 2011. Heat stress in wheat during reproductive and grain-filling phases. Crit. Rev. Plant Sci. 30 (6), 491–507.
Ferris, R., Ellis, R.H., Wheeler, T.R., Hadley, P., 1998. Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. Ann. Bot. 82 (5), 631–639.
Filion, M., Dutilleul, P., Potvin, C., 2000. Optimum experimental design for Free-Air Carbon dioxide Enrichment (FACE) studies. Glob. Change Biol. 6, 843–854.
Fischer, R.A., 1985. Number of kernels in wheat crops and the influence of solar radiation and temperature. J. Agric. Sci. (Camb.) 105, 447–461.
Fischer, R.A., 2011. Wheat physiology: a review of recent developments. Crop Pasture Sci. 62 (2), 95–114.
Fitzgerald, G.J., Tausz, M., O'Leary, G., Mollah, M., Tausz-Posch, S., Seneweera, S., Mock, I., Lowe, M., Partington, D., Argall, R., McNeil, D., Norton, R.M., 2016. Elevated atmospheric [CO_2] can dramatically increase wheat yields in semi-arid environments and buffer against heat waves. Glob. Change Biol. 22, 2269–2284.
Fokar, M., Blum, A., Nguyen, H.T., 1998. Heat tolerance in spring wheat. II. Grain filling. Euphytica 104 (1), 9–15.
Gan, Y., Stobbe, E.H., 1995. Effect of variations in seed size and planting depth on emergence, infertile plants, and grain-yield of spring wheat. Can. J. Plant Sci. 75 (3), 565–570.
Houshmandfar, A., Fitzgerald, G.J., Armstrong, R., Macabuhay, A.A., Tausz, M., 2015. Modelling stomatal conductance of wheat: an assessment of response relationships under elevated CO_2. Agric. For. Meteorol. 214, 117–123.
Houshmandfar, A., Fitzgerald, G.J., Macabuhay, A.A., Armstrong, R., Tausz-Posch, S., Low, M., Tausz, M., 2016. Trade-offs between water-use related traits, yield components and mineral nutrition of wheat under free-Air CO_2 Enrichment (FACE). Eur. J. Agron. 76, 66–74.
IPCC, 2014. In: Field, C.B., Barros, V.R., Dokken, D.J., Mach, K.J., Mastrandrea, M.D., Bilir, T.E., Chatterjee, M., Ebi, K.L., Estrada, Y.O., Genova, R.C., Girma, B., Kissel, E.S., Levy, A.N., MacCracken, S., Mastrandrea, P.R., White, L.L. (Eds.), Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 1132.
Isbell, R., 2002. The Australian Soil Classification, revised 1st edition. CSIRO Publishing, Collingwood, Victoria.
Kaur, K., Gupta, A.K., Kaur, N., 2012. Effect of drought and sowing time on stem reserve mobilization and sink activity in wheat (*Triticum aestivum* L.). Agrochimica 56 (1), 42–54.
Kimball, B.A., Morris, C.F., Pinter, P.J., Wall, G.W., Hunsaker, D.J., Adamsen, F.J., LaMorte, R.L., Leavitte, S.W., Thompson, T.L., Matthias, A.D., Brooks, T.J., 2001. Elevated CO2, drought and soil nitrogen effects on wheat grain quality. New Phytol. 150 (2), 295–303.
Kiniry, J.R., 1993. Nonstructural carbohydrate utilization by wheat shaded during grain-growth. Agron. J. 85 (4), 844–849.
Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B., 2017. lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26.
Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., Rogers, A., Long, S.P., Ort, D.R., 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: Six important lessons from FACE. J. Exp. Bot. 60 (10), 2859–2876.
Long, S.P., Ainsworth, E.A., Rogers, A., Ort, D.R., 2004. Rising atmospheric carbon dioxide: plants face the future. Annu. Rev. Plant Biol. 55 (1) 591-C-3.
McMaster, G.S., LeCain, D.R., Morgan, J.A., Aiguo, L., Hendrix, D.L., 1999. Elevated CO2 increases wheat CER, leaf and tiller development, and shoot and root growth. J. Agron. Crop. Sci. 183 (2), 119–128.
Mollah, M., Norton, R., Huzzey, J., 2009. Australian grains free-air carbon dioxide

enrichment (AGFACE) facility: design and performance. Crop Pasture Sci. 60 (8), 697–707.
Narayanan, S., Prasad, P.V.V., Fritz, A.K., Boyle, D.L., Gill, B.S., 2015. Impact of high nighttime and high daytime temperature stress on winter wheat. J. Agron. Crop. Sci. 201, 206–218.
Nie, G.Y., Long, S.P., Garcia, R.L., Kimball, B.A., Lamorte, R.L., Pinter, P.J., Wall, G.W., Webber, A.N., 1995. Effects of free-air CO_2 enrichment on the development of the photosynthetic apparatus in wheat, as indicated by changes in leaf proteins. Plant Cell Environ. 18 (8), 855–864.
Nuttall, J., Brady, S., Brand, J., O'Leary, G., Fitzgerald, G.J., 2012. Heat waves and wheat growth under a future climate. Capturing Opportunities and Overcoming Obstacles in Australian Agronomy, Proceedings of the 16th ASA Conference, 14-18 October 2012, Armidale, Australia. URL. https://www.agronomy.org.au/.
Palta, J.A., Fillery, I.R.P., 1995. N-application enhances remobilization and reduces losses of preanthesis-N in wheat grown on a duplex soil. Aust. J. Agric. Res. 46 (3), 519–531.
Parsons, R., Weyers, J.D.B., Lawson, T., Godber, I.M., 1997. Rapid and straightforward estimates of photosynthetic characteristics using a portable gas exchange system. Photosynthetica 34 (2), 265–279.
Pearson, P.N., Palmer, M.R., 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. Nature 406 (6797), 695–699.
Perez-Martin, A., Flexas, J., Ribas-Carbó, M., Bota, J., Tomás, M., Infante, J.M., Diaz-Espejo, A., 2009. Interactive effects of soil water deficit and air vapour pressure deficit on mesophyll conductance to CO_2 in *Vitis vinifera* and *Olea europaea*. J. Exp. Bot. 60, 2391–2405.
R Core Team, 2017. R: a Language and Environment for Statistical Computing. URL. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Rashid, M.A., Andersen, M.N., Wollenweber, B., Korup, K., Zhang, X.Y., Olesen, J.E., 2018. Impact of heat-wave at high and low VPD on photosynthetic components of wheat and their recovery. Environ. Exp. Bot. 147, 138–146.
Sadras, V., Dreccer, M.F., 2015. Adaptation of wheat, barley, canola, field pea and chickpea to the thermal environments of Australia. Crop Pasture Sci. 66 (11), 1137–1150.
Schnyder, H., 1993. The role of carbohydrate storage and redistribution in the source-sink relations of wheat and barley during grain-filling – a review. New Phytol. 123 (2), 233–245.
Shanmugam, S., Kjaer, K.H., Ottosen, C.O., Rosenqvist, E., Kumari Sharma, D., Wollenweber, B., 2013. The alleviating effect of elevated CO_2 on heat stress susceptibility of two wheat (*Triticum aestivum* L.) cultivars. J. Agron. Crop. Sci. 199 (5), 340–350.
Sharkey, T.D., 2005. Effects of moderate heat stress on photosynthesis. Plant Cell Environ. 28, 269–277.
Sild, E., Younis, S., Pleijel, H., Selldén, G., 1999. Effect of CO_2 enrichment on non-structural carbohydrates in leaves, stems and ears of spring wheat. Physiol. Plant. 107 (1), 60–67.
Simpson, R.J., Lambers, H., Dalling, M.J., 1983. Nitrogen redistribution during grain-growth in wheat (*Triticum-aestivum* L): IV. Development of a quantitative model of the translocation of nitrogen to the grain. Plant Physiol. 71 (1), 7–14.
Slafer, G.A., Rawson, H.M., 1995. Base and optimum temperatures vary with genotype and stage of development in wheat. Plant Cell Environ. 18 (6), 671–679.
Smart, D.R., Chatterton, N.J., Bugbee, B., 1994. The influence of elevated CO_2 on non-structural carbohydrate distribution and fructan accumulation in wheat canopies. Plant Cell Environ. 17 (4), 435–442.
Stone, P., Nicolas, M., 1994. Wheat cultivars vary widely in their responses of grain yield and quality to short periods of post-anthesis heat stress. Funct. Plant Biol. 21 (6), 887–900.
Stone, P., Nicolas, M., 1996. Effect of timing of heat stress during grain filling on two wheat varieties differing in heat tolerance. II. Fractional Protein Accumulation. Funct. Plant Biol. 23 (6), 739–749.
Sun, A.Q., Somayanda, I., Sebastian, S.V., Singh, K., Gill, K., Prasad, P.V.V., Jagadish, S.V.K., 2018. Heat stress during flowering affects time of day of flowering, seed set, and grain quality in spring wheat. Crop Sci. 58 (1), 380–392.
Tahir, I.S.A., Nakata, N., 2005. Remobilization of nitrogen and carbohydrate from stems of bread wheat in response to heat stress during grain filling. J. Agron. Crop. Sci. 191 (2), 106–115.
Talukder, A.S.M.H.M., McDonald, G.K., Gill, G.S., 2013. Effect of short-term heat stress prior to flowering and at early grain set on the utilization of water-soluble carbohydrate by wheat genotypes. Field Crops Res. 147 (0), 1–11.
Taub, D.R., Wang, X., 2008. Why are nitrogen concentrations in plant tissues lower under elevated CO_2? A critical examination of the hypotheses. J. Integr. Plant Biol. 50 (11), 1365–1374.
Tausz-Posch, S., Norton, R.M., Seneweera, S., Fitzgerald, G.J., Tausz, M., 2013a. Will intra-specific differences in transpiration efficiency in wheat be maintained in a high CO2 world? A FACE study. Physiol. Plant. 148 (2), 232–245.
Tausz-Posch, S., Borowiak, K., Dempsey, R.W., Norton, R.M., Seneweera, S., Fitzgerald, G.J., Tausz, M., 2013b. The effect of elevated CO_2 on photochemistry and antioxidative defence capacity in wheat depends on environmental growing conditions – a FACE study. Environ. Exp. Bot. 88, 81–92.
Tausz-Posch, S., Dempsey, R.W., Seneweera, S., Norton, R.M., Fitzgerald, G.J., Tausz, M., 2015. Does a freely tillering wheat cultivar benefit more from elevated CO_2 than a restricted tillering cultivar in a water-limited environment? Eur. J. Agron. 64, 21–28.
Telfer, P., Edwards, J., Bennett, D., Kuchel, H., 2013. Managing Heat Stress in Wheat, Australian Grain Technologies (AGT). Roseworthy Campus.
Thilakarathne, C.L., Tausz-Posch, S., Cane, K., Norton, R.M., Fitzgerald, G.J., Tausz, M., Seneweera, S., 2015. Intraspecific variation in leaf growth of wheat (*Triticum aestivum*) under Australian Grain Free Air CO_2 Enrichment (AGFACE): is it regulated through carbon and/or nitrogen supply? Funct. Plant Biol. 42 (3), 299–308.
Trevelyan, W.E., Harrison, J.S., 1952. Studies on yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. Biochem. J. 298–303.
Uhlen, A.K., Hafskjold, R., Kalhovd, A.H., Sahlstrom, S., Longva, A., Magnus, E.M., 1998. Effects of cultivar and temperature during grain filling on wheat protein content, composition, and dough mixing properties. Cereal Chem. 75 (4), 460–465.
van Herwaarden, A.F., Richards, R., Angus, J., 2003. Water-soluble carbohydrates and yield in wheat. 11th Australian Agronomy Conference.
Wahid, A., Gelani, S., Ashraf, M., Foolad, M.R., 2007. Heat tolerance in plants: an overview. Environ. Exp. Bot. 61 (3), 199–223.
Wang, X., Cai, J., Liu, F., Jin, M., Yu, H., Jiang, D., Wollenweber, B., Dai, T., Cao, W., 2012. Pre-anthesis high temperature acclimation alleviates the negative effects of post-anthesis heat stress on stem stored carbohydrates remobilization and grain starch accumulation in wheat. J. Cereal Sci. 55 (3), 331–336.
Wickham, H., 2009. ggplot2: Elegant Graphics for Data Analysis Springer-Verlag. New York. .
Winzeler, M., Dubois, D., Nosberger, J., 1990. Absence of fructan degradtion during fructan accumulation in wheat stems. J. Plant Physiol. 136 (3), 324–329.
Wollenweber, B., Porter, J.R., Schellberg, J., 2003. Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. J. Agron. Crop. Sci. 189 (3), 142–150.
Xue, G.-P., McIntyre, C.L., Jenkins, C.L.D., Glassop, D., van Herwaarden, A.F., Shorter, R., 2008. Molecular dissection of variation in carbohydrate metabolism related to water-soluble carbohydrate accumulation in stems of wheat. Plant Physiol. 146 (2), 441–454.
Zadoks, J.C., Chang, T.T., Konzak, C.F., 1974. Decimal code for growth stages of cereals. Weed Res. 14, 415–421.
Zamani, M.M., Nabipour, M., Meskarbashee, M., 2014. Stem water soluble carbohydrate remobilization in wheat under heat stress during the grain filling. Int. J. Agric. Biol. 16 (2), 401–405.
Zhang, Y.P., Zhang, Y.H., Xue, Q.W., Wang, Z.M., 2013. Remobilization of water soluble carbohydrates in non-leaf organs and contribution to grain yield in winter wheat under reduced irrigation. Int. J. Plant Prod. 7 (1), 97–116.
Ziska, L., 2008. Three-year field evaluation of early and late 20th century spring wheat cultivars to projected increases in atmospheric carbon dioxide. Field Crops Res. 108 (1), 54–59.